Effects of Beta-Carboline Alkaloids of Peganum Harmala on Induced Rat Ileum Contractions

Amjad T. Shatarat1,*, Sawsan Abuhamdah2,3, Eman Alefishat3,4, Mohamed K. Al-Essa3, Rima Altaweel R1, Faisal Mohammed5, Darwish Badran1, Hanan Jafar1

ABSTRACT

Peganum harmala L., Zygophyllaceae popularly known as Wild Syrian rue, a well-known plant in folk medicine for many pharmacological uses including antispasmodic activity. Chemical composition of the plant showed that the most important constituents of this plant are beta-carboline alkaloids such as harmalol, harmaline, and harmine. In this work, we aimed to evaluate the effects of these three major harmala alkaloids on induced rat ileum contractions, induced by acetylcholine, BaCl2, and KCl. Of these three harmala alkaloids, harmalol and harmaline produced a concentration-dependent spasmolytic activity, which was found to be reversible (i.e. disappeared after tissue wash-up). Both alkaloids inhibited acetylcholine and KCl-induced ileum contractions but BaCl2 -induced contractions were only inhibited by harmalol but not harmine. Harmine did not show any inhibitory activity.

Key words: Peganum Harmala L.; β-Carbolines alkaloids; Harmine; Harmaline; Harmalol; Rat; Ileum; Spasmolytic.

INTRODUCTION

Peganum harmala L. also known as Syrian rue, that belongs to the Family of Zygophyllaceae, is a perennial herbaceous, glabrous plant, which grows in a dry grassland with high temperature in the summer and low temperature in the winter (or grows in a desert climate) and sandy soils. It may grow up to 100 cm1 with white flowers between June and August. The flowers are single, small with five petals. Each flower tends to develop into a fruit2, the fruits are green and change to orange-brown when mature, the fruits are also preserved in a capsule with three chambers, each capsule contains more than 50 small black-brown triangular seeds.3,4

Peganum harmala L. is widely used as a medicinal plant that can be found in North Africa, Mediterranean, the Middle East, Pakistan, India and southern parts of Iran, and recently has also been found to grow in Australia and southwest of America.5 Through history, the usage of P. harmala was traced, for example, in west Asia this plant used as a talisman against voodoo and evil eye6, in the middle east it is used as psychoactive substance for spiritual experiences and it is also found to be used as a hallucinogenic aid by ancient Persian and Indian.7 In traditional medicine, P. harmala has been used for the treatment of different conditions, like asthma, lumbar, colic, jaundice and to stimulate menstrual flow.7 However, in recent pharmaceutical studies, P. harmala found to have an antispasmodic, antimicrobial, emmenagogue and abortive effects8, blocking different types of intestinal calcium channels9, mono amine oxidase inhibition and anti-depressant effect10,11, analgesic12, vasorelaxant activity against phenylephrine-induced contraction of isolated rat aorta13, anti-platelet aggregation effects14, hallucinergic, and anti-neoplasm effect.15-18

The active pharmacological compounds of P. harmala are alkaloids, β-carbolines (like harmine, harmaline, harman and harmalol) which can be found in high levels in seeds, roots and least found in leaves and stems.17 Quinazoline alkaloids (such as vasicine and vasicinone) have also been identified.17 The major alkaloids of P. harmala can be found in seeds and roots, harmaline (also known as harmidine) and harmine (banisterine) have the same pharmacological action but harmine (banisterine) is considered as less toxic. The active alkaloids of halmal seeds are the monoamine oxidase inhibitor A (MAOI-A) compounds, for this reason the popularity of this plant among western psychonauts as a psychoactive drug.19

Previous study suggested that P. harmala alkaloids, β-carbolines (harmine, harmaline, and harmalol) have a relaxant effect on smooth muscle of the trachea contracted by KCl20; in another study, β-carbolines (harmaline) found to inhibit acetylcholine induced ileal contraction.20 Therefore, the present study was carried out to examine the spasmolytic activity of the three major harmala alkaloids i.e., harmine, harmaline, and harmalol on the isolated rat ileum preparations induced by different stimulants like, acetylcholine (Ach), BaCl2, and KCl.

MATERIALS AND METHODS

Drugs and chemicals

All chemicals used in this study were of analytical grade and were obtained from St. Louis, MO, USA.

Harmaline, harmine, harmalol, acetylcholine chloride (≥ 98.0% purity) were also purchased from Sigma Aldrich and a stock solutions was prepared on daily basis. Kreb’s solution was prepared fresh, just before the experiment and consisted of (in mm): NaCl (118.1), KCl (4.7), CaCl₂ (2.5), MgSO₄ (1.2), NaHCO₃ (25) and glucose (5.6).

Experimental animals
Three-month-old male Wister rats (250–300 g) were obtained from the animal facilities of the Faculty of Medicine, The University of Jordan. The animals were housed under standard husbandry conditions, which included an ambient temperature 20–22 °C and a 12/12 h light/dark cycle, with free access to food and water. All animal experiments were conducted in concordance with the University of Jordan’s “Regulations and Ethical Guidelines for the Care and Use of Laboratory Animals”.

Ileum-tissue isolation and sample mounting
Four segments of each rat ileum were used on each day of the experiment. The animals were anesthetized by ether and sacrificed. The abdominal cavity was opened by a midline incision aseptically. One cm from the flexure of the intestine was cut and the segment of the ileum was dissected out and placed in oxygenated Krebs solution at (pH 7.4) at room temperature. The ileum tissue was carefully flushed out with freshly prepared Krebs solution maintained at 37°C bubbled with gas mixture of 95% O₂ and 5% CO₂. From a resting tension of 2 g, isotonic contractions, elicited by KCl, BaCl₂ and Ach, were recorded using Radnoti, 159901A, the isometric force transducers with computerized data acquisition system. Before the start of the experiment, all preparations were allowed to equilibrate for at least 30–45 min, during which Krebs solution was replaced twice. To study the spasmolytic effect of harmaline, harmine or harmalol, contractile agents such as ACh-chloride, potassium chloride (KCl) or BaCl₂ were added to the organ bath in the absence (control) or in the presence of various concentrations of harmaline, harmine, or harmalol.

Statistical analysis
Mean and standard error of the mean (S.E.M.) values were calculated for each group of results and the significance of difference between the means was calculated using one-way analysis of variance (ANOVA) followed by Dunnett’s test. Differences were considered statistically significant when P < 0.05. Harmaline, harmine or harmalol -evoked spasmolytic effect were expressed as a percentage of relaxation from spasmon induced plateau contraction from the concentration – response curve by data fitting using GraphPad Prism version 5.02 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS
Effects of harmaline, harmine or harmalol on ACh induced ileum contraction
A single application of Ach (3 × 10⁻⁵ M) evoked 100% contraction (Figure 1). Harmine had no significant effect on baseline tension but harmaline and harmalol inhibited the Ach induced contractile responses, harmaline at 5 µM and 10 µM (**P < 0.05) and harmalol at 5 and 10 µM (**P < 0.0001) respectively. These inhibitory effects could be seen within 10 min of contact with the tissue and were maintained as long as these alkaloids present in the bath.

Figure 1: Effect of Harmaline, Harmalol and Harmine (0.5–10 µM) on Ach induced contractions of rat isolated ileum preparations. Data are mean ± S.E.M (n = 8, 8, 12 respectively in each group) and expressed as % of control tension.

**P < 0.05, ***P < 0.0001, ANOVA followed by Dunnett’s test.

Effects of harmaline, harmine or harmalol on KCl induced ileum contraction
The effect of harmalol, harmaline, and harmine on KCl induced contractions was investigated using the ileum tissue that was exposed to KCl (60 mM) solution for 30 minutes before the experiments were commenced. The percentage inhibition of contraction induced by KCl in the presence of each concentration of the alkaloid was calculated. As in the case of Ach-induced contraction, the ileum preparation was
exposed to increasing concentration of harmine without showing any effect. However, harmaline at (1 and 5 µM) concentrations induced inhibition in contraction that was significant. Data are mean ± S.E.M and expressed as % of control tension. *P < 0.05 ANOVA followed by Dunnett's test (Figure 2).

**Effects of harmaline, harmine or harmalol on BaCl₂ induced ileum contraction**

As shown in (Figure 3), harmalol was the only alkaloid that produced significant inhibitory effect on BaCl₂, reducing the maximum induced contraction at 10 µM, **P < 0.01 (ANOVA followed by Dunnett's test).** No significant decrease was observed when the preparation was pretreated with harmaline or harmine.

---

**Figure 2:** Effect of harmaline, Harmalol and Harmine (0.5-10 µM) on KCl induced contractions of rat isolated ileum preparations. Data are mean ± S.E.M (n= 4,8,7 respectively in each group) and expressed as % of control tension. *P < 0.01, ANOVA followed by Dunnett's test.

**Figure 3:** Effect of Harmaline, Harmalol and Harmine (0.5-10 µM) on BaCl₂ induced contractions of rat isolated ileum preparations. Data are mean ± S.E.M (n= 4, 8,8 respectively in each group) and expressed as % of control tension. **P < 0.01, ANOVA followed by Dunnett's test.

**Figure 4:** Solvent effect on rat ileum contraction

The alkaloids were dissolved in 5% dimethylsulfoxide (DMSO) in all conducted experiments therefore to determine whether the solvent alone was able to inhibit contractions. Five percent DMSO has been added to some preparations without harmalol, harmaline and harmine. The solvent had no effects on KCl induced contraction of the ileum (Figure 4).

**DISCUSSION**

Certain similarities as well as distinct difference in the antispasmodic response to the three harmala alkaloids observed on rat ileum contraction.
contractions. Harmalol, harmaline exhibited similar effect, whereas harmine was not active. Increasing concentrations of harmalol and harmaline were found to reduce the intensity of rat ileum contractions (Figures 1-3).

This finding agrees that a methoxy group on the indole nucleus, and the C3-C4 double bond (Harmine) is important for antispasmodic activity. Harmalol in which a 7-hydroxy group replaces the methoxy group of harmaline and no double bond at C3-C4 produces good antispasmodic effect evoked by Ach, BaCl₂ and KCl. Harmaline in which 7-methoxy group, similar like harmine but lack C3-C4 double bond also appears to be active. While harmine with both methoxy group on the indole nucleus, and the C3-C4 double bond had no effect. The order of inhibitory effect of the contraction induced was harmalol > harmaline > harmine.

The Ach, KCl and BaCl₂ are commonly used spasmogens to detect the spasmyloytic activity of intestinal smooth muscles of different gastrointestinal membrane depolarization, increases action potential and muscle contraction. 25,26 Earlier studies have suggested that in the intestinal smooth muscles, Ach, KCl and BaCl₂ caused contraction through excitation of nerve motor function of the gastrointestinal tract via mechanisms that most probably involve Ca²⁺ ions. However, this effect can be shown at higher concentrations.

### REFERENCES


GRAPHICAL ABSTRACT

ABOUT AUTHORS

Amjad T. Shatarat, MD, Ph.D. Associate professor at the Department of Anatomy and Histology, School of Medicine, The University of Jordan. Currently, chairman of Anatomy department. Graduated from Crimea State Medical University/Russia in 2000. Then finished the PhD program from The University of Nottingham/UK in 2011. Teaching Anatomy, Histology and Embryology Courses to Medical, Dental, Pharmacy, Rehabilitation and Nursing students. Focusing on Research activities related to blood vessels, role of different medicinal plants on the smooth muscles, anatomical variations and radiology. He Participated in many National and International conferences and had more than 20 publications.

Dr. Sawsan Abuhamdah is a Jordanian registered pharmacist, has completed her PhD from Durham University, UK and postdoctoral studies from Granada Medical School, Department of Pharmacology, associate professor at the faculty of pharmacy, University of Jordan, and now working at college of Pharmacy, Al Ain University, Abu Dhabi, UAE.

Dr. Abuhamdah has published many original research articles in peer-reviewed journals and participated in the preparation of many symposium abstracts. Dr. Abuhamdah is a member of the British Pharmacological Society (BPS), British Neuroscience Association (BNA), and Jordan Pharmaceutical Association.
Dr. Alefishat received her Ph.D. from the University of Nottingham, UK in 2011, her thesis focused on the cardiovascular system. She works as an associate professor and assistant dean for PharmD Program and Hospital Affairs at School of pharmacy in the University of Jordan. Dr. Alefishat published several papers in peer reviewed international journals, and have collaborations with colleagues from the United States and the United Kingdom. Her research is in the area of circadian disruption and its relationship with insulin resistance, vitamin D deficiency, cardiovascular and metabolic diseases, and inflammation.

Mohamed K. Al-Essa, MD. Ph.D. Associate professor in the Department of Physiology and Biochemistry at the Faculty of Medicine/The University of Jordan. Graduated from Medical College of Virginia Commonwealth University/USA in 1999. Teaching Medical Physiology Courses to Medical, Dental, Pharmacy, Rehabilitation and Nursing students. Focusing on Research activities related to regulatory mechanisms, stem cells and development of assay methods. He Participated in many National and International conferences and had more than 15 publications.

Rima K. Altaweel, research assistant in the Department of Anatomy and Histology at the Faculty of Medicine/ The University of Jordan. Graduated from University of Jordan, rehabilitation faculty/Jordan in 2012. Then finished the Master program of Anatomy and Histology from The University of Jordan/Jordan in 2019. Assist in teaching Anatomy practical labs to Medical, Pharmacy, and Rehabilitation students. Focusing on Research activities related to Anatomy, Histology and Rehabilitation field.

Faisal I. Mohammed is a professor of Physiology at the University of Jordan. Currently, he is the Chairman of the department of physiology and biochemistry. He graduated from University of Jordan medical school, then finished his PhD program from St. Louis University, USA. He participated in teaching physiology to all medical professional schools’ students. His special interests are topics related cardiovascular physiology and smooth muscle physiology.

Darwish Badran is a professor of Anatomical Sciences at the University of Jordan. Currently, he is the Dean of the Faculty of Medicine at the Hashemite University, Jordan. Graduated from Glasgow University, 1992. He participated in teaching Anatomy, Histology, Embryology and Neuroanatomy to Medical and Dentistry Students for more than 25 years. He participated in developing Anatomical Sciences curricula in many Medical Schools. He participated in many National, Regional and International conferences and has more than 60 published papers. His special interests are topics related to Clinical Anatomy and Histology.

Hanan Jafar holds a PhD in "Stem Cell Biology", an MSc in "Anatomy and Histology", and a DDS in "Dental Sciences" from the University of Jordan. She is jointly appointed as faculty member at the School of Medicine and Assistant Director at the Cell Therapy Center (CTC), University of Jordan.